An introduction to restoration genetics.

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1. Why is genetic diversity important?

All living organisms carry a genetic blueprint. This is so regardless of whether they are plants, animals, or fungi, whether they are short- or long-lived, and whether they reproduce sexually or clonally. Therefore, to the extent that restoration deals with living organisms, genetics are part of the picture. Although the basic principles underlying restoration genetics may be familiar, to date surprisingly little attention has been devoted to genetic considerations in restoration practice. The purpose of this Restoration Science and Policy Paper is to outline some considerations that restoration designers and managers should be aware of, and to identify more detailed resources that may be useful in practice.

a. Genotypes partly determine organisms' physical form and function.

Biologists refer to two basic expressions of variation, the genotype and the phenotype. The *genotype* is the genetic code of an organism; in organisms with nucleated cells (most multi-cellular plants, animals, and fungi), the essential code is found in the nucleus of each cell. Additional genetic codes reside in other components of the cell, such as mitochondria (in animals) and chloroplasts (in plants). An organism's genotype consists of a large number of *genes* (50,000-100,000 in a typical vertebrate), which can be at multiple sites (*loci*) on chromosomes. Genes have a variety of functions, the most important of which is to code for the production of specific amino acids, which are ultimately used to synthesize proteins. In most higher plants and animals (but not fungi, bryophytes, and many marine invertebrates), the adult phase has two copies of each gene, one derived from each parent. When the two copies are the same, the individual is *homozygous* for that gene; if the two copies are different, the individual is *heterozygous* for that gene. The various forms of a gene are referred to as *alleles*; when these forms are identical across a population, the gene is considered *monomorphic*, and if more than one allele exists the gene is considered *polymorphic.*

Phenotype is the expression of these genes as a living organism in a particular environment, and is influenced by environmental context at every level from the cell to the whole organism. It is frequently very difficult to separate variation in an organism's traits with a genetic basis from traits that are phenotypically variable; mistakes are commonly made both ways (assuming a genetic basis when observed variation is really phenotypic plasticity; underestimating subtle genetic effects on traits that are assumed to be simply "phenotype"). For

example, a plant growing in a poor environment might end up having a small and stunted phenotype, despite having "good" genes. This does not mean that genetic variation is not important to the fitness of individuals or populations. Both the environment and the genes will ultimately contribute to restoration success, but steps to ensure an optimal genetic makeup of a population are commonly overlooked.

Examples abound in the scientific literature illustrating how genetic composition affects the form and function of organisms (Hamrick, Linhart, and Mitton 1979; Hedrick 1985; Primack and Kang 1989; Rehfeldt 1990; Allen, Antos, and Hebda 1996; Hartl and Clark 1997). In fact, the recognition of genetic variation among individuals was a primary insight that led to the formulation of evolutionary theory as we know it today (Freeman and Herron 1998). Genes regulate body size, shape, physiological processes, behavioral traits, reproductive characteristics, tolerance of environmental extremes, dispersal and colonizing ability, the timing of seasonal and annual cycles (*phenology*), disease resistance, and many other traits (Raven, Evert, and Eichhorn 1986). Thus, to ignore genetic variation in ecology is to ignore one of the fundamental forces that shape the biology of living organisms. From a restoration perspective, organism and population genetics are fundamental considerations in the design, implementation, and expectations of any project, whether or not explicit consideration is given to the genetic dimension.

b. Genetic diversity helps organisms cope with current environmental variability.

Organisms exist in environments that vary in time and over space. Such variation is often described in terms of the *natural* or *historic range of variability* (NRV, HRV) in environmental conditions such as weather, disturbance events, resource availability, population sizes of competitors, etc. (White and Walker 1997). If a group of organisms (say, a population of species X) were to live in a completely stable physical and biological environment, then a relatively narrow range of phenotypes might be optimally adapted to those conditions. Under these circumstances, Species X would benefit more by maintaining a narrow range of genotypes adapted to prevailing conditions, and allele frequencies might eventually attain equilibrium. By contrast, if the environment is patchy, unpredictable over time, or includes a wide and changing variety of diseases, predators, and parasites, then subtle differences among individuals increase the probability that some individuals and not others will survive to reproduce -- *i.e.*, the traits are "exposed to selection." Since differences among individuals are determined at least partly by

genotype, population genetic theory predicts (and empirical observation confirms) that in variable environments a broader range of genetic variation (higher heterozygosity) will persist (Cohen 1966; Chesson 1985; Tuljapurkar 1989; Tilman 1999).

Examples of traits with a genetic basis for tolerance of environmental variation important in restoration work include tolerance of freezing, drought or inundation, high or low light availability, salinity, heavy metals, soil nutrient deficiencies, and extreme soil pH values in plants; resilience to fluctuating temperature, dissolved oxygen, and nutrient availability in aquatic organisms; and resistance to novel diseases in all groups of organisms (Huenneke 1991). For example, if all individuals in a population are the same genotype with limited drought tolerance, then a single climatic event may destroy the entire population. Plant populations often include individuals with a range of flowering or emergence times. For instance, Great Basin shrub populations include individuals that leaf out and flower over a period of weeks, increasing the likelihood of persistence of the population through periods of unusually early or late growing conditions. Knapp *et al.* (2001) documented flowering periods in a population of individual blue oak trees and found that trees initiated flowering over a period of a month in the spring. Such variability could potentially be adaptive, since it is more likely that at least some trees in the population will flower during warm sunny periods when wind pollination is most successful.

A diverse array of genotypes appears to be especially important in disease resistance (Schoen and Brown 1993; McArdle 1996). Genetically uniform populations (such as highly inbred crops) are famously vulnerable to diseases and pathogens, which can (and do) decimate populations in which all individuals are equally vulnerable. Such uniformity also predisposes a population to transmit disease from one individual to another: instead of having isolated diseased individuals, nearly every individual may be exposed to disease by direct contact or proximity. More diverse populations are more likely to include individuals resistant to specific diseases; moreover, infected individuals occur at lower density, and thus diseases or pathogens may move more slowly through the population.

Finally, genetic variation is a factor in competition among individuals in real ecological communities. Traits with a genetic basis such as flower size are key factors in competition among individuals. Among animals, behavioral traits may regulate interspecific competition. Since organisms make energetic or life history tradeoffs among traits (for example, allocating energy between growth and reproduction), genetic variability is an important factor in how

populations function (Koyama and Kira 1956; Thompson and Plowright 1980; Fowler 1981; Gurevitch 1986; Goldberg 1987; Manning and Barbour 1988; Welden, Slauson, and Ward 1988; Grace and Tilman 1990; Tilman and Wedin 1991; Pantastico-Caldas and Venable 1993; Wilson and Tilman 1993; Delph, Weinig, and Sullivan 1998).

c. Diversity within populations reduces potentially deleterious effects of breeding among close relatives.

In addition to its adaptive value at the population level, genetic variation (or its lack) within individuals can affect their survival and performance. When both copies of a gene (in a diploid organism) are identical (*i.e.*, when an individual is *homozygous* at that gene or locus), the expression of that gene may include traits that are less beneficial to survival or reproduction in particular circumstances. This may lead to physiological or behavioral problems of genetic origin, such as malformed physical structure, poor biochemical balance, improper organ formation and function, altered social behavior, and susceptibility to disease (Chai 1976).

Homozygosity at key gene loci is a common result of *inbreeding*, which is sexual reproduction among closely related individuals. Small populations and species that do not disperse well (or are constrained by a fragmented landscape from exchanging genes with other populations) can be particularly susceptible to *inbreeding depression*, which is reduced overall survival and reproduction of organisms with low heterozygosity. Inbreeding depression arises from a variety of causes, including expression of unfavorable or deleterious alleles, and often leads to lower survival and birth or reproductive rates (Templeton 1991; Meffe and Carroll 1994; Husband and Schemske 1996).

Heterozygosity is not always beneficial, nor does inbreeding always have adverse effects. In some circumstances, a population may be so well adapted to its local circumstances that introducing alleles from other populations actually reduces its performance (*outbreeding depression*) (Templeton 1991). Paradoxically, organisms can experience outbreeding depression for some traits, while suffering the effects of inbreeding depression for other characters. Many organisms (particularly plants) have evolved breeding strategies, such as self-pollination, that allow successful persistence in small populations without apparent short-term effects of inbreeding depression.

d. Genetic diversity is the primary basis for adaptation to future environmental uncertainty.

Finally, genetic variation holds the key to the ability of populations and species to persist over evolutionary time through changing environments (Freeman and Herron 1998). No organism can predict the future (and evolutionary theory does not require them to), nor can any organism be optimally adapted for all environmental conditions. Nonetheless, the current genetic composition of a species influences how well its members will adapt to future physical and biotic environments.

Consider, for example, the response of a population of plant Species A to a period of rapid climate change. Changes in climate are reflected locally in altered annual rainfall and its seasonal distribution, changes in annual mean maximum and minimum temperatures and their seasonality, changes in prevailing wind direction and speed, and many other factors. These changes cause the zone of suitable climate for Species A to shift geographically. Populations with a diverse array of optima for these climatic conditions (*i.e.*, some individuals that are slightly more cold or heat tolerant, etc.) are more likely to persist, because some individuals will survive the new conditions and reproduce. If suitable climate zones shift across the landscape, seeds of some individuals may be dispersed into the new location and find good conditions for growing. In this manner, the population can "migrate" across the landscape over generations. By contrast, populations that have a narrower range of genotypes (and are more phenotypically uniform) may simply fail to survive and reproduce at all as conditions become less locally favorable. Such populations are more likely to become *extirpated* (locally extinct), and in extreme cases the entire species may end up at risk of extinction. For example, the Florida Yew (*Torreya taxifolia*) is currently one of the rarest conifer species in North America. But in the early Holocene (10,000 years ago), when conditions in southeastern North America were cooler and wetter than today, the species was probably widespread. For reasons that are not completely understood, *T. taxifolia* failed to migrate northward as climate changed during the Holocene. Today, it is restricted to a few locations in the Apalachicola River Basin in southern Georgia and the Florida panhandle.

As the *T. taxifolia* story illustrates, once species are pushed into marginal habitat at the limitations of their physiological tolerance, they may enter an "extinction vortex," a downward cycle of small populations, reduced genetic variability, reduced ability to adapt to novel

conditions, leading to further reductions in population size, and so on (Shaffer and Samson 1985; Gilpin and Soulé 1986). Reduced genetic variability is a key step in the extinction vortex.

2. How is genetic diversity distributed in natural populations?

There are genetic differences among individuals within most (but not all) populations of plants and animals. There are also differences among populations across the range of each species. In this section we review some basic patterns of how genetic diversity of species is distributed, or *partitioned*.

a. Monomorphic and polymorphic alleles.

By definition, individuals within a given species share some percentage of alleles; otherwise, they would not be considered members of the same species. This shared (or common) portion of the gene pool includes two basic classes of genes. Genes that are monomorphic (*i.e.*, $A = 1$, where A is the number of alleles per locus) within a species are common to all populations, and indeed essentially to all individuals. (Technically, "monomorphic" genes are defined as those for which the frequency of the most common allele is $\geq 99\%$; thus, some variation may be present even at these loci). The monomorphic proportion of the total genome varies among taxonomic groups, typically in the range of 85% in animals and 50% in plants (Hedrick 1985; Hartl and Clark 1997).

The remainder of loci (on average, 15% in animals and 50% in plants) is polymorphic ($A > 1$), varying among individuals in a population, and among populations within a species. These loci are of most concern to restoration, since polymorphic loci may or may not be adequately represented depending on the design and execution of a restoration program.

b. Diversity within and among populations of a single species.

The genetic profile of whole populations typically varies from place to place across a species range. These differences may arise as the result of chance occurrences, such as the genetic composition of dispersing individuals that create a new population (*founder effect*), or changes in allele frequencies that result from chance matings in very small populations (*genetic drift*) (Primack and Kang 1989; Templeton 1991; Meffe and Carroll 1994; Eckert, Manicacci, and Barrett 1996; Husband and Schemske 1996). Differences among populations can also arise

systematically, especially if the environment in various places exposes individuals to different optima for survival and reproduction (*fitness*). For these and other reasons, populations often diverge from one another in their genetic composition. Such divergence is especially strong and rapid when there is little *gene flow* between populations (*e.g.,* limited dispersal of seeds or pollen, or limited movement of animals across physiographic barriers). Over evolutionary time, such among-population genetic differences can accumulate and eventually result in the development of a new species (*allopatric speciation*). Indeed, "populations" are defined as much (or more) by patterns of mating and gene flow as by the physical distribution of individuals, although the two are often closely related (Hartl and Clark 1997).

Each species distributes its genetic diversity (one measure of which is the total of all alleles at all loci) in a pattern reflecting both its biology and its history (Wright 1965; Nei 1975). For example, nearby populations of plants that are pollinated by bees may share many alleles because genes (packaged in pollen grains) can flow easily between sites. Such species may have fewer unique alleles in each population, so populations tend to be genetically similar. By contrast, there may be less gene flow among populations of species that are pollinated by ground-dwelling flightless beetles, or whose heavy fruits fall to the ground in the vicinity of the parent tree. Gene flow can also be obstructed by physical barriers (*i.e.*, topography or habitat that a pollinator, disperser, or migrating individual cannot cross), as well as by disturbance (Levin 1981; Slatkin 1987).

Even if variation within a population is low, there may be considerable variability *among* populations. Imagine Species X, a plant that lives in high mountaintop alpine areas, in which every individual on a given mountain is nearly identical genetically to others. In this case, individuals in Population 1 will all be nearly identical to each other; individuals in Population 2 will also be identical to each other, but because populations are very isolated from one another, they may all be very different from all the individuals in Population 1; and so on. Under these circumstances, most of the variation in Species A is *among* populations; within-population variation is low or nonexistent.

Now imagine a contrary example. Species B lives in tallgrass prairie, where suitable habitats are relatively close to one another (or even continuous), and dispersal among populations is common. Here we might find a great deal of variation among individuals in each population (reflecting the benefits of variation discussed above), but because gene flow is high

among sites, most populations are similar (that is, most polymorphic alleles are widely distributed). This illustrates a species with a high proportion of variation distributed *within* populations, while among-population variation is relatively small.

A variety of measures are used to quantify the distribution of genetic variation among individuals within populations, and among populations. These measures are the basis for describing how genetic variation is *partitioned* within species. Differences among populations are commonly quantified by the use of one of several statistics, including Wright's inbreeding coefficient (F_{ST}) and Nei's coefficient of gene variation (G_{ST}). These indices are functions of how heterozygosity is partitioned within and among populations, based on differences in allele frequencies (Wright 1969; Nei 1975; Chai 1976; Wright 1978). Where p_i is the frequency of the ith allele, heterozygosity (H) = $1 - \sum p_i^2$ for {i = 1...n} for populations within a larger sample. Then, the proportion of total variation that is distributed among populations (G_{ST}) is $\frac{H_T - H_S}{H_T}$, or equivalently, $1 - (H_S / H_T)$, where H_S and H_T are the mean heterozygosity within populations and in the entire species respectively. Values for these statistics can often (although not often enough!) be found in the population genetic literature, so restorationists do not have to generate them. Values of F_{ST} and G_{ST} vary from 0 to 1 (Nei 1975; Hedrick 1985; Crow 1986; Hartl and Clark 1997). G_{ST} in particular has a number of useful properties: it can be used for one or many loci, mutation rates do not alter the statistic significantly, the exact number of populations need not be specified, and the statistic is relatively responsive to changes in allele frequencies in time. Although they have important conceptual differences, in practice F_{ST} and G_{ST} are used in similar fashion as indices of genetic difference among populations (Crow 1986).

Species genetic structure can be modeled in a variety of ways. The simplest is a "finite island" conceptual model, in which alleles can flow between any pair of populations. In this case, the key parameters are the migration rate and *effective population size* (Crow 1986). However, the assumption that any two populations are equally likely to exchange alleles is probably unrealistic. Instead, a "stepping stone" model can be used, in which alleles spread by "stepping" from one population to an adjacent one (Wright 1969; Crow 1986).

In practical terms, G and F statistics tell us whether the majority of genetic variation is distributed among or within populations. In species with low G_{ST} (approaching 0), the majority of variation is found *within populations*; individuals within populations are likely to be

genetically different, but each population contains the same complement of alleles in similar frequencies Where G_{ST} is high (approaching 1), individuals within a population are relatively similar but *populations* are significantly different. Most species fall somewhere in between these extremes.

The distribution of genetic variation within and among species is strongly linked to life-history traits, particularly dispersal and *reproductive mode* (Hamrick and Godt 1990; Hamrick et al. 1991). Species that disperse genes (in plants, this includes both pollen and seeds) widely and frequently will tend, other things being equal, to have lower G_{ST} (*i.e.,* populations will be more similar). Even a moderate rate of gene movement among populations (one individual every a few generations) can "link" the gene pools of two populations. Mutation and drift (chance selection of genotypes, especially likely in small populations) can also lead to changes in allele frequencies, although in general these forces are believed to act more slowly than dispersal and selection.

c. How is genetic variation detected and measured?

A variety of methods exist for the assessment of genetic variation (Schaal, Leverich, and Rogstad 1991). Traditionally, genetic variation was inferred by the shape (*morphology*) or growth responses of organisms. Such approaches often use *common garden experiments* or *reciprocal transplants* to distinguish genotypic and phenotypic variation. In this approach, individuals that appear phenotypically different (or that grow in different environments) are placed in a common environment. This eliminates the environmental component of variation; presumably, the remaining variation has a genetic basis.

While these methods can be useful, they measure higher-order effects of genetic variation (*i.e.*, expression in the whole organism), and hence address genetic variation only indirectly. Moreover, the confounding effects of phenotypic variation are often difficult to separate from underlying genetic differences among organisms. In practical terms, it is often not possible to know whether organisms that look different are actually different genetically, or whether their differing phenotypes reflect the influences of environment or chance in development.

Since the 1960's the most widely applied method of estimating genetic differences among individuals is *enzyme electrophoresis* (also referred to as *starch gel electrophoresis*, including both *allozyme* and *isozyme* analysis). Electrophoresis detects variation in amino acids that are early products of gene *translation* (the process by which a cell translates a copy of its

genetic code in messenger-RNA into amino acids). Since there may be multiple biochemical pathways leading to the synthesis of a single amino acid or enzyme, electrophoresis is relatively conservative in its estimates of genetic variation among individuals (Hamrick, Linhart, and Mitton 1979). Enzyme electrophoresis remains the most widely used method of estimating genetic variation, and literally thousands of studies have been conducted on a wide range of organisms. Electrophoretic analysis permits individuals to be distinguished from one another based on enzyme variation. These differences among individuals can be compiled to generate statistics about the degree of variation within and among populations (§2.b), a key consideration in restoration ecology.

In the past decade, powerful methods have developed and emerged to detect variation in DNA itself, not simply its products (Karp, Isaac, and Ingram 1998). While a bewildering variety of tools and techniques may be encountered in the literature, they all have in common the ability to reconstruct the sequence of nucleotides in the DNA molecule, arguably the most basic level of genetic variation (Britten 1986; Clegg 1990). The conceptually simplest (although experimentally most laborious) technique is *gene sequencing* -- that is, the exact reconstruction of the complete nucleotide sequence. Part of the difficulty in interpreting DNA sequences is that "genes" in a functional sense may be distributed over several sections of a chromosome; there are also large sections of all chromosomes that do not code for any known function, or that are redundant or *regulatory* (influence the transcription, translation, or function of other genes). Thus, unlike enzyme analysis, much of the information in a DNA sequence may be of little relevance to understanding ecologically significant genetic variation. Since in theory every sexually-produced individual is likely to be unique, DNA sequences can be almost too variable to detect the kinds of patterns required for restoration work. However, new analytical techniques allow DNA sequence variation to be interpreted in meaningful ways. Complete gene sequences have been complied for relatively few organisms, and generally only a few individuals each, although these numbers are increasing all the time.

It is important to note that isozymes and genetic markers may not reflect traits under strong natural selection. For example, Knapp and Rice ((1998) evaluated patterns of variation in a native grass using both quantitative traits and isozymes, and compared these to geographic distance and climate. They found overall that isozymes did not reflect an adaptation to climate, whereas the quantitative traits did. In a restoration context, this suggests that climate may in

some cases serves as more useful guide for collecting and reintroduction zones that genetic markers *per se.*

3. How do genetic questions enter into restoration?

The genetic principles outlined above have a direct bearing on the practice of ecological restoration (Falk and Holsinger 1991; Young and Clarke 2000). In this section we summarize how restoration practitioners and researchers can (and should) take population genetics into account.

a. Accuracy and functionality of restored populations.

Sophisticated restoration practitioners recognize that the starting pool of genetic variation to be used is a critical element in design and implementation. However, opinions abound on how to select a suitable source pool for a restoration project (Landis and Thompson 1997).

The current debate often confuses two related, but distinct issues: the *accuracy* ("authenticity") of a restoration project, and its *functionality* (Clewell, 2000). A reintroduction project is genetically *accurate* ("authentic") if it replicates the original gene pool of the population it replaces. If the original population has been destroyed, then perfect accuracy is strictly speaking impossible, because some alleles were probably unique to that population (*i.e.*, $G_{ST} < 1$). A restoration can be accurate and function poorly; it can also be inaccurate but functional.

Since no reintroduction is perfectly accurate genetically, the question then becomes: "How close is close enough?" To this there can be no absolute ecological answer, since not all variation is of adaptive significance. Moreover, variation among populations is a continuous variable: two populations can differ by one allele or thousands. If the motivation is an ethical commitment to fidelity to the historic distribution of genotypes, the line can be drawn anywhere. The best practice is to estimate and report the degree of genetic accuracy based on available information, so that others studying a project can take authenticity into account.

How well a reintroduced population functions is a very different matter. Here the concern is not so much whether specific genotypes have been re-established, but rather how well a restored population will work in terms of persistence, resilience, and stability. Since many adaptive traits have a genetic basis, reintroduction material that performs (survives, grows,

reproduces) well may come from a site with similar ecological conditions, but not necessarily close geographically (Knapp and Rice 1998; Procaccini and Piazzi 2001). Moreover, if functionality is the main objective, then a range of genotypes can be introduced, allowing selection (differential survival and reproduction) to sort out the best for the site. If this approach is taken, there may be considerable attrition, which can be accounted for in initial sampling (§3.c).

b. Geographic location of source material for (re)introduction.

Perhaps no single genetic issue is more intensely debated in restoration circles than the location of source populations: "Where should material for restoration come from?" Interest in population genetic variation often arises in the context of asking whence source material should come. In other words the restorationist is concerned not only with the degree of variability but its particular geographic distribution.

The most common approach is to specify a geographic (and often elevation) range, within which source material can be confidently collected. This is essentially a "space-for-genotype" substitution: we expect that populations near one another, and growing in similar conditions, will be more similar genetically due both to ecotypic variation and the effects of gene flow. Geographic distance is a reasonable first approximation of genetic distance, assuming that genetic diversity most likely obeys the "distance decay of similarity" in broad outline (Nekola and White 1999). In population genetic terms, this favors the "stepping stone" model (§2.b) of gene flow. On public lands in the western United States, commonly-applied guidelines suggest that material for outplanting be collected from within 1,000 ft. elevation bands and 100 miles lateral distance of the planting area (elevation bands vary from 500-3,000 ft for different agencies) (see § 4, *Resources*). As noted above (§ 2.c), climatic zones may also offer a better first approximation of genetic distance between populations than simple geographic distance (Knapp and Rice 1998; ONPS 2001).

Unfortunately, there are no simple distance rules that apply equally to all species, because species vary in gene flow among populations, population size, and the resulting distribution of diversity (G_{ST}). For some species (*e.g.*, self-fertilizing plants in small, isolated patches of habitat, or fishes in isolated stream reaches), each site may reflect a unique local adaptation, and the geographic range of suitable genotypes can be very small (a few km^2). Other species (for example, species with wind dispersed pollen and seeds) with higher rates of gene flow, and those

with larger effective populations, are probably less differentiated over the landscape and can be collected from much wider ranges. Most species have dispersal curves that are leptokurtic (*i.e.*, with long tails), meaning that a few seeds or offspring in each generation may travel far beyond the majority; while few in number, these long-range dispersers may play a critical role in helping species to adjust their ranges in periods of climate and vegetation change. For each species, the restorationist must ask: "How widely does this species disperse its genes under normal conditions, and what factors (distance, geographic barriers, habitat types) influence where genes can spread?" This uncertainty is reflected in the wide range of recommendations for suitable collecting zones, which range over more than three orders of magnitude from 100 meters to 100 miles.

The issue of geographic range for source material cannot be separated from the distinction made earlier between historical accuracy and functionality. If historical accuracy (authenticity) *sui generis* is the primary concern, then the range of potential collecting sites is governed most strongly by historical patterns of dispersal, and the collecting radius will be very small. If population function is the primary concern, on the other hand, then the net may be cast over a wider area (although still focusing on similar ecological communities). This is because populations that are widely separated in space may nonetheless occupy similar ecological settings, and by selection may have developed genotypes that are similar in key ecological traits. If the main goal is population function, then it is probably more important to derive source material from large, diverse populations than from small, genetically depauperate sites, even if the latter are closer geographically. Of course, it is always possible to mix genotypes from different populations, and to let selection sort out the variation: this is, after all, exactly what nature does. Mixing of genotypes may be particularly suitable if existing "populations" are in fact isolated and reduced remnants of formerly widespread and interconnected groups. Re-combining fragmented populations genetically is appropriate as the geographic scale is ecologically realistic, and should not be done indiscriminately. Mixing too broadly (*i.e.*, combining genotypes from very diverse ecological settings) can result in too many individuals that are poorly adapted for the new given environment (high genetic load). Some geneticists advocate the use of regional mixtures -- composite collections of genotypes, all of which are at least moderately well adapted to a given environment (Knapp and Dyer 1997; Lesica and Allendorf 1999).

There are valid arguments on both sides of the authentic *vs.* functionality debate. On one hand, there is little question that the gene pools of many remnant native populations have been seriously eroded, so that what occurs today is a small remnant of the original diversity. Small gene pools are more prone to inbreeding, as well as random genetic change from drift. Populations that formerly exchanged genes regularly may have also become genetically isolated by habitat fragmentation. In such cases, a good argument can be made to bring together genetic material from several nearby populations, in effect replicating the natural (but now disrupted) processes of gene flow. In addition, some restoration sites may be so heavily disturbed (*i.e.* mine tailing reclamation areas) that the most "local" population may no longer be the one best adapted to the growing environment. In such cases, a wide diversity of genotypes can increase the chances that at least some plants will have the genetic composition to survive.

It is possible, however, that genotypes from outside the apparent current range may perform too well -- that is, swamp the local genotype. If remnant native populations of the species being introduced already exist at a site, additional genetic considerations reinforce the importance of the scale of collection. Introduced populations may hybridize with the existing native population, introducing new genes (genetic pollution) and potentially negatively affecting genetic integrity (Rieseberg 1991; Glenne and Tepedino 2000). If a few poorly adapted individuals make it into the existing native population, it might be argued that natural selection will eventually remove the deleterious genes. However, the issue is partly one of numbers; with commercial seed production of many native species, restorationists now have the tools to dump large volumes of seed into ecosystems. If the number of poorly adapted, non-local propagules is large in relation to the number of local native types, the chance of matings between the well adapted and the poorly adapted plants will increase (especially in cross-pollinating species), thereby potentially swamping the native population and diluting the local genes. Progeny from such matings may additionally experience outbreeding depression (*i.e.* poor survival and growth in relation to the parents). Carefully chosen introductions, with genotypes similar to the existing native population, can avoid these negative impacts.

Take-home messages: Since no single rule applies to all situations, the most important recommendation is to use explicit and reasoned criteria in selecting source populations. To summarize the issues presented here:

1. Species vary in their dispersal rates and distances, and hence in the degree of genetic

differentiation among populations. These differences are often closely correlated with life-history attributes particular to each species that affect rates and patterns of gene flow. Therefore, guidelines are inherently species-specific.

2. The restorationist must decide if historical accuracy or functionality is the primary objective. True historical accuracy can be difficult to determine if existing populations are remnants of a formerly widespread range. Moreover, most natural populations experience a degree of genetic change over time and space. However, even where the emphasis is on functionality, historical reference conditions are essential to "anchor" restoration work within a natural range of variability.
3. Geographic distance (*i.e.*, the "space-for-genotype" substitution) is a reasonable, but crude, substitute for patterns of gene flow among populations. However, in a disrupted landscape the most local remnant populations may be genetically reduced, and may not have genotypes that correspond to the conditions under restoration. Moreover, if populations are strongly selected to local habitat conditions, then habitat similarity may outweigh distance as a selection criterion. Local and regional climatic and soil zones can be useful criteria for obtaining material for reintroduction, since these will often represent plants adapted to the conditions at the restoration site.
4. As a source of material for reintroduction, large, genetically diverse source populations are generally preferable to small, limited populations even when the latter are closer to the restoration site. It may be preferable to combine material from several suitable sites, to capture a wider array of genotypes that can succeed in the new location. Such "regional mixtures" should draw on populations within a similar ecological zone (climate, soil) to the restoration site, to avoid too large a proportion of individuals that may not be well adapted to the new conditions.
5. Small local populations can be "swamped" by non-native genotypes if a species already exists at a restoration site. If existing populations are to be augmented, the number of individuals introduced from other locations should not be so large as to overwhelm the local gene pool, particularly if there is evidence of local adaptation.

c. Sampling the diversity of source populations.

The initial gene pool for a restoration project is inevitably limited by the diversity of the original sample. While other genes may enter the project area over time (for instance, by

migration of individuals, dispersal of gametes, or additional reintroduction measures), the starting pool of genetic diversity will govern the performance of a reintroduced population for a long time. Hence, the diversity of the original source collection is a critical consideration in restoration.

A variety of guidelines have been developed for sampling wild populations of plants and animals for breeding and reintroduction (Center for Plant Conservation 1991; Guerrant 1992; BGCI 1994; Guerrant 1996; IUCN/SSC 1998). These guidelines address four fundamental sampling issues:

1. How many populations will be sampled to create the source pool?
2. How many individuals will be sampled from each population?
3. Will samples be collected all at once, or over a period of years?
4. What is the probability of a collected sample surviving to establishment?

Number of populations: In most species, the cumulative amount of genetic variation captured increases as successive populations are added to the sample. However, since populations have some measure of similarity ($0 < G_{ST} < 1$), each additional population added to a sample collects some alleles that are new to the sample, and some that are already present from previous samples. As the number of populations sampled increases, the *marginal diversity rate* decreases (that is, fewer and fewer novel alleles are captured), and the *cumulative diversity function* approaches an asymptote. For a pool of populations sampled at random, there comes a point at which further sampling across populations provides little or no additional genetic benefit (Falk 1991; Falk and Gibbs *In prep.*).

The number of populations at which this occurs is related strongly to the measure of differentiation among populations, G_{ST}, which we have already encountered. When G_{ST} is high, populations are markedly different from one another, and more should be sampled to capture the maximum total diversity. When G_{ST} is low, most populations are similar, and sampling from only a few will capture most of the diversity that exists(provided that the statistic was generated using populations across the full range of the species). Of course, it is always worth remembering that a significant fraction of most genomes is monomorphic, and is therefore captured in the first population, if not the first individual.

There can be no hard and fast rules for the number of populations to be sampled across all taxa. In a recent survey of 13 plant species, the sample for some species was saturated for

common isozyme alleles after just 3-5 populations (20% of the total), while in other species the cumulative diversity curve was still rising after all populations (as many as 15-30) were included in the sample (recall, however, that isozyme loci may be less variable among populations than other loci under strong selection) (Falk and Gibbs *In prep.*). Rare or uncommon alleles accumulate in the sample at a slower rate than do common alleles, so if it is suspected that important adaptations occur at low frequency (for instance, because of recent environmental change), then it is advisable to sample a larger number of sites. Further research is ongoing in this area; in the meantime, G_{ST} or some comparable measure of population differentiation is a suitable guide for the sampling strategy.

One exception to this general null model is for populations that are strongly differentiated along habitat lines (*e.g. ecotypes*). If the reintroduction area is unusual habitat for a species, then it is worth sampling any populations that occur in similar conditions, to increase the likelihood of capturing alleles that offer adaptive benefit.

Number of individuals to sample within populations. The underlying theoretical basis for sampling multiple individuals within a population is that populations are rarely truly *panmictic* (that is, with completely randomized breeding). In plants, a large proportion of mating occurs between neighboring individuals, even when pollination occurs via an animal vector. In animal populations, a wide range of behavioral adaptations exists that commonly concentrates breeding success in a few individuals at any given time. The result is that populations are not genetically homogeneous; to capture their genetic diversity adequately, multiple individuals need to be sampled.

Again, a number of general guidelines have been released on this topic, and again, there can be no hard and fast "magic numbers" that apply across taxa (Brown et al. 1990; Brown and Briggs 1991). Plant studies evaluated by the Center for Plant Conservation (Center for Plant Conservation 1991) and its colleague institutions suggest that anywhere from 10-50 individuals should be sampled per population, with the understanding that there is bound to be considerable redundancy among these samples. Sampling of propagules (seeds, vegetative shoots) has less impact on the source population, although there is often lower success associated with these life stages compared to outplanting established plants (Guerrant 1992; Guerrant 1996).

Number of years during which samples will be collected. For many species, an adequate sample can be collected in a single year. However, for many others, collecting may need to be distributed over a number of years. Reasons for multiple-year collecting include:

- Variable reproductive output (masting), where large numbers of individuals are reproductive or large numbers of offspring are produced only in certain years;
- Small populations or species with low reproductive rates, where it is important to avoid depleting the pool of new individuals;
- Annual organisms, for which gene pools may be significantly different from year to year, either by selection or by chance; and
- Limited capacity to handle (store, propagate, release) collected material.

Probability of a collected sample surviving to establishment. In the end, what counts in a reintroduction is the number of individuals in the new population as well as their diversity (Menges 1991). However, less than 100% of the samples (seeds, cutting, eggs, adults) collected in the field will survive to establishment. Attrition occurs at every step along the way: during the collecting process, transportation, storage, propagation/curation, and outplanting/release. High initial mortality rates are often observed in reintroduced populations, often continuing for several years (Brown and Briggs 1991).

A simple calculation can help to account for attrition of collected samples. Let *Ps* represent the survival probability of a collected sample *s*, and *N* the number of individuals desired in the final restored population. Then we account for attrition during the reintroduction process by collecting N/Ps samples in the field. For example, suppose that trees dug for transplant have an ultimate survival rate of 40% from initial collecting to the third year of a reintroduction project, and that we are trying to establish a population of 50 trees. Correcting for attrition, an initial collection of (50/.40), or 125 individuals, will give us a good chance of ending up with the final population size we wish (Brown and Briggs 1991).

d. Genetic diversity within reintroduced populations.

As discussed above, breeding among closely related individuals can lead to expression of unfavorable traits that compromise an organism's ability to survive and reproduce. Individuals resulting from inbreeding in populations where this did not occur naturally from highly inbred lines can have stunted growth, altered behavior, deformed morphology, poor physiological

function; such individuals are also more likely to be reproductively sterile. Hence, the genetic origin of individuals used in a restoration project can influence whether a project succeeds or fails. If source collections are made from diverse, naturally occurring populations, the probability of breeding among close relatives is reduced. A more diverse population may include individuals that can tolerate a wider range of conditions. If, on the other hand, all individuals in a restored population are genetically similar, and the total population size is not large (fewer than 100 individuals), the probability is great that their offspring will be homozygous at some loci, and thus have reduced fitness. A genetically narrow population may be able to survive only in a narrow range of conditions. While genetic diversity is not always associated with *ecological amplitude*, in general the correlation is positive (Huenneke 1991).

In addition to affecting how well a restored population succeeds in current environmental conditions, genetic diversity may affect performance over time. Important ecological traits, such as tolerance of disturbance or climatic extremes often have a strong genetic basis. Such traits need to be considered not only in terms of conditions at the time of reintroduction, but in light of the natural range of variability over many years or generations. If the goal of restoration is to establish self-sustaining populations (Pavlik 1996), then the range of conditions to which the population and its offspring will be exposed is a vital consideration. Genetically uniform populations may do well one year when conditions are favorable for their genotype, and then fail the next, even though the range of environmental variation is within normal limits. Such failures are even more likely in the case of less common extreme events (Gaines and Denny 1993). Recalling from § 1.b and 1.d that environmental conditions vary within an envelope of natural variation, it is safe to assume that most reintroduced populations will be exposed to a wide range of conditions over time. Hence, the genetic diversity of reintroduced populations is a non-trivial consideration for their long-term persistence (Montalvo et al. 1997).

It is not uncommon in restoration work for large numbers of individuals to be released or outplanted, a large proportion of which fail to survive beyond 2-3 years. This means that restoration releases are subject both to the *founder effect* (the result of the initially limited gene pool) and then further *genetic bottlenecks* as effective population size diminishes due to mortality (Robichaux, Friar, and Mount 1997). If the initial outplanting or release is itself genetically uniform (*e.g.*, hundreds or thousands of clonally-produced plants), the resulting gene

pool in the field can be quite narrow. Such apparently large populations are, in genetic terms, very small (Brown and Briggs 1991).

Of course, genetically narrow populations occur in nature, often resulting from similar forces (founder events and bottlenecks). For example, aspen (*Populus tremuloides*) and many species in the Iridaceae (*Iris*, *Lilium*) are often found in large clumps of many *ramets* of very few *genets*, or even just one. Natural populations of species where sexual (as opposed to vegetative or parthenogenic) reproduction dominates are less likely to be genetically homogeneous (Meffe and Carroll 1994).

The effects of genetic homogeneity in a reintroduced population may not be immediately evident, but over a period of years the population may have lower rates of growth, survival and reproduction, and may persist less successfully through periods of natural environmental variability.

What you can do: Restorationists should be aware of the level of genetic diversity that they are working with. Above all, restoration planners and managers should understand how the plants and animals they use were generated. Ask your nursery or whoever collected and propagated your seed source (or your breeder, in the case of animals) what methods were used, and what steps were taken to assure the presence of a suitably wide range of genotypes. While clonally-produced flats of thousands of identical plants may appear to offer short-term advantages (as they do for agricultural crops) of predictable response to current growing conditions, in the long run (again as with crops) such populations may be less likely to persist and succeed in the face of disease, competition, and climate variability. Of course, knowing the methods by which individuals or material was produced provides genetic information only by inference; it is unfortunately not common to have good genetic data for reintroduction efforts.

e. Genetic diversity among reintroduced populations.

The same principles apply to diversity among population diversity. Most species have a degree of differentiation among populations (*i.e.*, $G_{ST} > 0$). Population geneticists debate whether these differences have adaptive value in any given case – that is, whether they are the result of selection – or whether they represent the effects of sampling error (founder effects, drift, mutation). Nonetheless, a reasonable precautionary position is to mimic the degree of genetic difference found in natural populations of a species when (re)introducing populations to the landscape.

Most species, however, have little or no published data on their among-population genetic diversity. The few surveys that have been done are invaluable sources of information, and restorationists should read and cite them whenever possible. In some cases studies of *congeners* can be used, although with the caveat that species that are closely related phylogenetically are not always similar ecologically.

4. Resources.

A vast literature exists on genetic diversity, its distribution in space and time, and its adaptive and ecological significance. A number of excellent texts on population genetics provide greater depth on a number of topics addressed briefly in this paper. Several of the growing numbers of papers on the topic of restoration genetics are listed in the literature cited section of this paper.

Conservation genetics is still is relatively small field, and restoration genetics is in its infancy. Restoration practitioners and scientists can contribute to the accumulation of knowledge in this area by treating your restoration projects as experiments, and publishing your results.

The Society for Ecological Restoration maintains an on-line *Directory of Restoration Expertise* at www.ser.org. The IUCN also has published a list of reintroduction practitioners (Soorae and Seddon 1998). Individuals listed in each of these resources may be helpful with restoration genetic issues.

a. Web sites and organizations.

Society for Ecological Restoration
1955 W. Grant Road, Suite 150
Tucson, AZ 85745 USA
Tel. (520) 622-5485
Fax. (520) 622-5491
Email: info@ser.org
www.ser.org

Plant Conservation Alliance
Bureau of Land Management
1849 C Street NW, LSB-204
Washington, DC 20240 USA
Tel. (202) 452-0392
http://www.nps.gov/plants/

Species Survival Commission Plants Programme
c/o International Union for Conservation of Nature (IUCN)
Rue Mauverny 28
CH-1196
Gland, Switzerland
Tel. 41/22/9990152
Fax 41/22/999015
http://www.iucn.org/themes/ssc/index.htm

Center for Plant Conservation
Missouri Botanical Garden
P.O. Box 299
St. Louis, MO 63166 USA
Tel. (314) 577-9450
http://www.mobot.org/CPC/

US Forest Service
Seed collecting guidelines:
http://www.na.fs.fed.us/spfo/rngr/pubs/np97/consider.htm

National Forest Genetic Electrophoresis Laboratory (NFGEL)
2480 Carson Road
Placerville, CA 95667 USA
Tel. (530) 622-1225
Fax (530) 622-2633
http://www.dendrome.ucdavis.edu/NFGEL/

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